Applications of Biofilm in the Degradation of Contaminants in Industrial Effluents-A Review

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Abstract

In this paper, it is presented a review about applications of biofilms in the degradation of contaminants in industrial effluents. Biofilms are typically comprised of water, microorganisms, extracellular polymeric substances (EPS), retained particles and dissolved and adsorbed substances. Water is the most significant fraction of the total mass of the biofilm, and it can vary from 70 to 95%. Polymeric substances represent around 70 to 95% of the organic material of the dry biofilm mass. The composition determines important properties of the biofilm, such as the adhesion force, elasticity, and adsorption capacity. Studies have shown that adhered cultures are less affected than suspended cultures by environmental changes: temperature, pH, nutrient concentration, metabolic products and toxic substances. In this paper, it is shown the application of a reactor with adhered biomass to the removal of BTX (benzene, toluene and o-xylene) compounds, in the fixed bed column with biofilm, for the different concentrations and feed flows. The results presented indicate a strong potential for the industrial use of the biodegradation system described herein, comprised of a metabolically active bacterial biofilm, supported on activated carbon, since there was bacterial reproduction in the biofilm and consumption of BTX compounds by this biomass.

Keywords: biofilms, microorganisms, treatment, effluents.

1. Applications of biological processes for the treatment of effluents

Applications of biological processes for the treatment of effluents involve the action of microorganisms, especially bacteria, fungi, algae and protozoan, for their degradation. The biodegradation process occurs through respiration or fermentation processes in which complex substances are transformed into simple compounds such as mineral salts, carbon dioxide, and methane. These processes are classified as aerobic and anaerobic. Aerobic and anaerobic processes can be divided into two large groups, considering the form of the
microorganisms: systems with suspended biomass and those with the biomass adhered to a support.

In suspended-biomass reactors the microorganisms are dispersed in the medium. Thus, the microorganisms (sludge) need to be separated from the liquid phase at the end of the process, with or without part of this sludge being returned to the reactor. This leads to the disadvantage that such reactors require large spaces for their installation.

The reactors with adhered biomass differ from those with suspended biomass in that they have distinct phases: a continuous liquid phase and a solid phase formed of microorganisms adhered to supports. The substrates must cross the liquid-biofilm interface and, by diffusion, and be transported along the biofilm, while the products formed must be transported in the opposite direction, allowing non-homogeneous conditions to occur in the reactor. Thus, the microorganisms of the biofilm can be subjected to different micro-environments.

The support medium, to which the microorganisms are adhered, can be fixed or mobile. In processes with fixed supports the microorganisms are immobilized on immobile supports, forming a permanent bed through which the effluent percolates (trickling filters) or circulates (biofilters or submerged filters). In processes with a mobile bed the microorganisms are immobilized on supports which can be moved mechanically (rotating biological contactor) or by hydraulic action (expanded bed, fluidized bed or airlift reactors).

Although biological processes are those most used in the treatment of organic effluents, extensive research has been carried out in order to minimize the drawbacks associated with their application, which include: instability of the system caused mainly by the variation in the concentration and composition of effluents from the various industrial processes; excessive production of biomass (sludge) since the degradation of the substrate aims at the formation of new cells, resulting in a new problem to be solved; and the large volume of biological reactors, due to the low activity of the microorganisms and large effluent flows, thus requiring a long retention time. Furthermore, the ever stricter standards required, in terms of the removal of nitrogen, phosphorous, micro-pollutants and odors, have also stimulated the development of new biotechnological processes, in relation to both suspended and adhered biomass.

In this regard, extensive research has been performed in relation to processes which employ adhered biomass, since these have some advantages over conventional processes (Lazarova and Manem, 1994; Ulson de Souza et al., 2008; Guelli U. de Souza et al., 2007; Kryst and Karamanev, 2001; Mello et al., 2010; Massalha et al., 2007; Ulson de Souza et al., 2011):

- Better performance in the removal of suspended material and dissolved pollutants;
- Coexistence of anaerobic and aerobic metabolic activity within the same biomass ecosystem;
- Higher metabolic activity attributed to the high concentration of nutrients adhered to the biofilm and the different interactions between microbial
species resulting in a high efficiency in the removal of the BOD (Biological Oxygen Demand);

- Application of a higher organic load, with a large area for mass transfer between phases, thus resulting in the use of more compact installations;
- High biomass concentrations can be attained which eliminates the need to recirculate the sludge and ensures a greater sludge age;
- Absence of risk of biomass leaching since with the biomass adhered to the support the reactor can operate at flow rates independently of the maximum specific growth rate;
- Reduced hydraulic retention time.
- Lower generation of sludge, resulting in less dependence on the sedimentation phase;
- Faster entrance into operational regime;
- Resistance to hydraulic load shocks and greater capacity to tolerate recalcitrant and toxic pollutants, as well as other adverse environmental conditions, due to the protector effect of the extracellular matrix (diffusion barrier) which can reduce the concentration of toxic compounds in the biofilm; and
- Reduction in installation and operation costs without affecting the performance of the treatment process.

Bioreactors can be operated with three types of process: batch, fed-batch or continuous.

In the batch process the reactor is loaded with the culture medium and inoculated with the microorganisms and the process proceeds until the nutrients are exhausted and/or the accumulation of the product of interest. The system is closed, that is, there is no addition of nutrients, the composition of the medium changes constantly due to cellular metabolism and the volume of the reactor medium remains constant. These bioreactors have the following advantages: easy operation and control, low risk of contamination, and suitability for short time periods. The disadvantages are: exhaustion of the culture medium and accumulation of toxic compounds or degradation of the product, preparation of the reactor between batches reduces the working time, and high costs (Ulson de Souza et al., 2011).

The fed-batch process initially functions as a batch system, but there is the addition of medium as the biodegradation proceeds. The feeding of medium or substrate can occur continuously (one stage) or in pulses (several stages). The reactor volume is variable. The advantages of this process are that is allows a high concentration of inducer substrates, impedes the effect of catabolic repression, maintains a low concentration of substrates which inhibit the formation of the product and allows high cellular concentrations to be obtained. The disadvantages are that it has a high risk of contamination and there is a greater need for process control (Ulson de Souza et al., 2011).

In the continuous process the medium is added continuously and the degradation products are continuously removed. The volume is maintained fixed or constant. In these systems an equilibrium state is reached, where the concentration of cells and nutrients is maintained constant and the product is produced continuously. The loss of cells and nutrients from the medium at the outlet is balanced by new cells formed when substrate is added. The important factors are the dilution or flow rate and the limiting substrate
concentration. The advantages are: greater volumetric productivity, control of the growth rate and maintenance of the cell metabolic activity for long periods and reduced loss of working time. The disadvantages are: greater risk of contamination (very long periods and open system), and the appearance of less productive mutants or genetic variants. These are the bioreactors most used in the treatment of residues (Ulson de Souza et al., 2011).

Fluidized bed reactors have been employed in aerobic processes (Lazarova and Manem, 1994; Ulson de Souza et al., 2008), and anaerobic process (La Motta and Cascante, 1996), for the removal of color (Sharma et al., 2004), denitrification (Coelho et al., 2010), and the removal of toxic compounds such as BTEX (Mello et al., 2010), among others.

The advantages of fluidized bed reactors is the large area for mass transfer between the phases and the lack of the bed clogging, the latter being a problem commonly associated with fixed bed reactors. On the other hand, many drawbacks and technical restrictions (control of bed expansion, film thickness and the system distribution and saturation of oxygen) hinder its application in industrial scale for aerobic processes (Lazarova and Manem, 1994).

The fluid dynamics aspects of a fluidized bed can be characterized by the following parameters:

- The minimum fluidization velocity depends on the density and size of the particles and the properties of the liquid, such as density and viscosity. The minimum fluidization velocity can be calculated through empirical correlations;
- Retention (hold-up) of the phases, which represents the volumetric fraction occupied by each phase; and
- Superficial gas and liquid velocities.

2. Biofilms

The formation of biofilms is a natural phenomenon through which microorganisms adhere to solid surfaces whenever they are in contact with water. The biofilm can be defined as a combination of microorganisms and extracellular products which adhere to a solid support, forming a voluminous and thick layer, with an external structure that is not completely regular and uniform. Its chemical composition, both inorganic and organic, varies according to the substrate composition (Costerton et al., 1995).

Biofilms are typically comprised of water, microorganisms, extracellular polymeric substances (EPS), retained particles and dissolved and adsorbed substances. Water is the most significant fraction of the total mass of the biofilm, and it can vary from 70 to 95%. Polymeric substances represent around 70 to 95% of the organic material of the dry biofilm mass. The composition determines important properties of the biofilm, such as the adhesion force, elasticity, and adsorption capacity (Nielsen et al., 1997).
The considerable interest in understanding the formation of biofilms, both with regard to their use and their destruction, is due to the fact that, in general, the biomass, when adhered, has greater activity, that is, it has higher rates of growth and substrate usage, in relation to the free biomass. This phenomenon is attributed, in some cases, to physiological changes which the adhered cells undergo and, in other cases, to the favoring of changes in the cellular environment due to an increase in the local concentration of nutrients and enzymes or to the selective effect of the extracellular polymeric matrix of the biofilm in relation to inhibitory or toxic substances. Studies have shown that adhered cultures are less affected than suspended cultures by environmental changes: temperature, pH, nutrient concentration, metabolic products and toxic substances (Farhadian et al., 2008).

Another important aspect is that the biofilm is an ecosystem, in which many species of microorganisms coinhabit and which are subject to interactions such as symbiosis or competition for space and nutrients. This is due to the constant environmental variation within the biofilm, for example, the concentration of the substrates, electron acceptors and intermediary products (Bishop, 1997).

According to Van Loosdrecht et al. (1995), Costerton et al. (1995), the development of the biofilm can be described in stages:

1. Latency or activation phase: Corresponds, in the reversible phase, to phenomena related to the adsorption of soluble materials (organic and inorganic nutrients) and particulates (microorganisms) at the surface of the support, which occurs through different forces: electrostatic forces, the Van der Waals attractive forces and weak forces of chemical and hydrophobic interactions; and, in the irreversible phase, the fixation, in which extracellular polymers play a fundamental role since it appears that these polymers act as ligands between the microorganisms and the support.

2. Exponential or dynamic phase: Is the phase in which the colonization of the support surface occurs and the growth rate is at the maximum. In this phase there is a considerable increase in the rate of polysaccharide and protein production and high consumption of the substrate.

3. Linear accumulation phase: Corresponds to a constant rate of biomass accumulation on the support.

4. Stabilization phase: In this phase the physical phenomena, such as the shearing and attrition forces, originating from the system fluid dynamics, begin to have an effect, causing the detachment of cells and hindering additional accumulation.

5. Stationary phase: In this phase, equilibrium between the detachment and growth of the biofilm cells can be observed, characterizing a permanent regime in relation to the solid phase.

6. Detachment phase: The detachment of the biofilm is a random phenomenon which is dependent on the behavior of the microorganisms directly adhered to the support.

According to Kryst and Karamanev (2001) and Massalha et al. (2007), the way in which a biofilm develops leads to important benefits for the microorganisms of which it is comprised, such as: an increase in the concentration of nutrients at the liquid-biofilm interfaces, since the polymeric matrix favors the adsorption of nutrient molecules; protection against harsh environmental factors such as fluctuation in pH, salt and heavy
metal concentrations, dehydration, shearing forces, aggressive chemical substances, bactericides, antibiotics and predators; possibility for the exchange of genetic material due to the long microorganism retention times; and facility of the development of micro-consortiums which allow the establishment of symbiotic relations as well as the use of substrates which are difficult to degrade.

The fixation of the microorganisms on the surface is the result of physical, chemical and biological phenomena, and the main factors affecting the biofilm formation and maintenance are (Herzberg et al., 2005; Van Loosdrecht et al., 1995):

1. Characteristics of the support: The properties of the solid surface are important in terms of the formation of the biofilm, in particular, the surface charge, roughness and hydrophobicity. Some authors consider the roughness as the most important factor (Van Loosdrecht et al., 1995), since it increases the fixation surface and protects it from the detachment caused by shearing, thus maintaining the microorganisms on the surface long enough for irreversible adhesion to occur and thereby allowing the formation of the biofilm.

2. Microorganism species: The formation of the biofilm varies according to the types of microorganisms present, due to their cell surface properties and their capacity to produce extracellular polysaccharides, which are responsible for maintaining cell aggregates.

3. Characteristics of the liquid phase: The characteristics of the liquid phase, which affect the formation of the biofilm, may be related to both its composition/concentration (organic and inorganic compounds) and its environmental conditions of pH and temperature, since both influence the microbial growth and the production of extracellular polysaccharides.

4. Fluid dynamics conditions: The formation and maintenance of the biofilm are influenced by the balance between the adhesion, growth and detachment of the cells. The detachment process is closely related to the shearing and attrition forces (resulting from collision between particles) which are a function of the fluid dynamics of the process.

Ulson de Souza et al. (2011) have presented three case studies relate to: the treatment of real effluent (Case study 1) from a textile plant using a mixture of sludge from an industrial and domestic effluent treatment station, with the inoculation carried out in a fed-batch regime; and the treatment of a synthetic effluent containing a textile dye (Case study 2) and containing BTX compounds (Case study 3), using sludge from a domestic effluent treatment station (anaerobic and activated sludge) in a fed-batch system for the textile dye and batch system for the BTX compounds. The results for the removal of BTX compounds by the biodegradation process, in the fixed bed column with biofilm, for the different concentrations and feed flows, are shown in Figure 1.

In Figure 1 it can be verified that the BTX compounds in the mixture are biodegraded by the previously adapted biofilm, with a more accentuated drop at the beginning of the column. Figure 1(a) shows that the o-xylene is the compound which is the most difficult for the microorganisms to biodegrade, followed by benzene and toluene, when the feed concentration of the BTX compounds is the same. It can also be observed that for the input concentration used (40 mg/L), the flow is high, since the concentration at the column output is higher than the discharge level permitted by the environmental bodies. Thus, for
Figure 1 – Concentration profile of BTX compounds along the column: (a) Initial concentration of 40 mg/L of each compound in the BTX mixture, input flow of 5.0 mL/min; (b) Initial concentrations of 78 mgB/L, 58 mgT/L and 29 mgX/L, input flow of 3.0 mL/min; (c) Initial concentrations of 92 mgB/L, 49 mgT/L and 35 mgX/L, input flow of 4.0 mL/min. Ulson de Souza et al. (2011).

A high input flow, the biodegradation is not complete, requiring a longer bioreactor, and the reverse situation occurs when the feed flow is decreased (Figure 1(b)) since even for an input concentration of BTX compounds above that of Figure 1(a), the concentration at the column output is lower than the initial value. This is due to the fact that the residence time of the BTX compounds in the column is lower for a greater flow, which inhibits the biodegradation process.

Formation of the biofilm on the surface of the activated carbon particles is shown through analysis of the bioparticle, using a Philips model XL 30 scanning electron microscope (SEM). The SEM results at magnifications of 500 and 5000 times are shown in Figure 2.

As can be observed in Figure 2, there was growth and immobilization of the microorganisms on the surface of the support, forming a thin biofilm with similar bacterial morphology on the external surface of these particles. It was observed that the distribution of microorganisms on the external surface of the particles was not homogeneous, with some
regions of large agglomerates of bacteria and others with a low concentration of microorganisms.

Through the protein analysis, using the method of Lowry, the biomass present in the bioreactors was quantified. Table 1 shows the average values for the quantity of protein in each bioreactor, in units of mg of protein per liter of bioreactor, and mg of protein per gram of support.

Table 1 - Quantity of protein in mg/L and in mg/g support, for the BTX compounds.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Benzene [mg/L]</th>
<th>Toluene [mg/L]</th>
<th>o-Xylene [mg/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>649.06</td>
<td>1125.98</td>
<td>117.90</td>
</tr>
<tr>
<td>Average [mg/g support]</td>
<td>11.440</td>
<td>18.179</td>
<td>2.023</td>
</tr>
</tbody>
</table>

From the values in Table 1 it can be verified that the quantity of protein was greater for the toluene, since this compound has a lower toxicity than the other two. In the case of o-xylene, the value was lower than those for benzene and toluene, showing that it was difficult for the biomass to adapt to these compounds, since the quantity of biomass placed in the reactor was the same and thus there was a lower microorganism reproduction rate in this medium.

Based on the results obtained for the removal of BTX compounds by biodegradation, it is possible to observe that the biofilm formed on the activated carbon was able to degrade
the BTX compounds, verifying that activated carbon is an effective alternative for the immobilization of biomass.

The results presented indicate a strong potential for the industrial use of the biodegradation system described herein, comprised of a metabolically active bacterial biofilm, supported on activated carbon, since there was bacterial reproduction in the biofilm and consumption of BTX compounds by this biomass (Ulson de Souza et al., 2011).

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References


